

**Diagnostic pigments as a means of tracking the distribution of algal functional groups
within the James River**

Subtask 1.2 Chl a, diagnostic pigments and the occurrence of harmful algae

Data Report

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by

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Abstract

Many phytoplankton species are potentially harmful and current chlorophyll criteria do not distinguish between algal species or groups. In the upper James River, cyanobacteria are the major bloom-forming group that exerts harmful effects while in the lower James River, dinoflagellates are the dominant group of harmful algae that produce blooms. Pigment analysis can be used to investigate and identify the distribution of major phytoplankton functional groups (e.g., diatoms, chlorophytes, cyanobacteria, and dinoflagellates), provide early indication of incipient blooms, provide a means of early detection of changes in taxonomic composition within the phytoplankton community and possible emergence of harmful algal species, and potentially advise mitigation actions. We related pigment concentrations with the distribution of major algal groups and with respect to environmental variables in the Lower James River estuary. Diagnostic pigments may offer a high-throughput method for augmenting taxonomic monitoring in aquatic systems and may allow modelers to distinguish chlorophyll impairments due to specific taxonomic groups.

Introduction

While chlorophyll *a* (Chl *a*) is a useful indicator of total algal abundance because this pigment occurs in most or all algal groups, it is not suitable for determining which algal groups dominate the plant biomass at any one time. Differentiating among algal groups is essential because algal groups differ in their nutrient preferences, biogeochemical function in ecosystems, nutritional value to higher trophic levels, and their impact on the environment. In the lower James River, is in many saline portions of estuaries (Sellner et al. 2001), dinoflagellates most often cause observed water quality impairments and many taxa within this group are potentially harmful. Harmful algal blooms (HABs) may be linked to impairments of designated uses even when there are no Chl *a* impairments. That is because many algae can result in impairments even if present at relatively low abundance. Consequently, it is useful to distinguish the abundance of different taxonomic groups. Algae contain a diverse suite of pigments and many species, including some potentially harmful species (e.g., the brown tide organism *Aureococcus anophagefferens* and the red tide organism *Karenia brevis*), contain unique pigments that allow their rapid identification within natural populations. Diagnostic pigment analysis has been used to assess the abundance of different groups of algae (e.g., dinoflagellates and cyanobacteria) and the abundance of particular algal species when there is a pigment biomarker.

Chl *a* is ubiquitous across major phylogenetic groups of algae, however, phytoplankton groups are characterized by their unique pigment compositions and specific pigments can be detected with great sensitivity. Empirical relationships between pigment concentrations and cell abundance have been used to rapidly ascertain shifts in community composition and species abundance over a range of seasons and biomass levels. If specific pigments can be linked to potentially harmful taxa, pigment analysis may also allow early detection of harmful algal blooms and associated impairments of designated uses.

In natural systems such as the James River estuary, we often observe that Chl *a*, cell abundance, and productivity are not well correlated. This is likely because Chl *a* is itself a physiological variable. Relationships between Chl *a* and other diagnostic pigments and phytoplankton cell abundance is being made under a range of environmental conditions including during harmful algal blooms (HABs). These data are needed to determine whether there are diagnostic pigments or pigment concentrations that might be early indicators of shifts in

phytoplankton community composition (Pinckney et al. 1998), bloom initiation or degraded water quality (Paerl et al. 2003, 2010).

Methods

In 2011 and 2012, we simultaneously sampled to measure phytoplankton cell abundance (Marshall/Egerton group), Chl *a* (Hampton Roads Sanitation District [HRSD]), particulate carbon (PC) and nitrogen (PN) (2012 only), and diagnostic pigment concentrations (via high performance liquid chromatography [HPLC]) over a range of salinities, seasons, and cell



Figure. 1. Surface sampling conducted for CMAP cruises in the lower James River including the meso- and polyhaline sections (JMSMH and JMSPH), the Elizabeth River polyhaline (ELIPH), and the Lafayette River mesohaline (LAFMH)

densities. Surface samples were taken in the lower James River including the meso- and polyhaline sections (JMSMH and JMSPH, respectively), Elizabeth River polyhaline (ELIPH), and Lafayette River mesohaline (LAFMH) (Fig. 1) during routine Chl *a* mapping as part of the Chlorophyll Monitoring and Assessment Program (CMAP). We did not receive samples from the tidal fresh or oligohaline James River from our collaborators. We collected samples for diagnostic pigment analysis, PC and PN, and cell abundance when Chl *a* exceeded our screening criteria of $15 \mu\text{g L}^{-1}$ and during periods when Chl *a* was $< 15 \mu\text{g L}^{-1}$. The CMAP sampling facilitated this paired sampling process in the lower James River because it provided a real-time readout of Chl *a* to guide sampling. A total of 1512 samples were collected during the CMAP program during 2011 and 2012. Of those, 505 samples were run and compared with microscopic cell counts. Of the 505 samples analyzed, 99 were from the

Elizabeth River, 107 from the Lafayette River, 104 from the JMSPH, and 195 from the JMSMH segments. Of the analyzed samples, 265 were designated as bloom samples by HRSD. The two dinoflagellates that contributed the majority to blooms were *Heterocapsa triquetra* (67 of the bloom samples) and *Cochlodinium polykrikoides* (157 of the bloom samples). Of the 505 samples analyzed, 181 samples had Chl *a* concentrations $< 10 \mu\text{g L}^{-1}$, 162 samples had Chl *a* concentrations between 10 and $25 \mu\text{g L}^{-1}$, 79 samples had $25\text{--}50 \mu\text{g L}^{-1}$ Chl *a*, and 82 samples had Chl *a* concentrations in excess of $50 \mu\text{g L}^{-1}$ (of those 37 had Chl *a* concentrations $> 100 \mu\text{g L}^{-1}$)

Samples for diagnostic pigment analysis were collected in the field using a peristaltic pump or a syringe and filtered through a glass fiber filter (Whatman GF/F). Samples were immediately frozen in liquid nitrogen and then transported in the liquid nitrogen dewer to ODU where they are transferred to a -80°C freezer. Diagnostic pigment analyses were conducted by HPLC (Van Heukalem and Thomas 2001, Hooker et al. 2005). PN and PC samples were filtered on GF/F filters and immediately frozen until analysis. Filters were dried (~ 2 days) at 40°C , pelletized in tin discs and analyzed using a Europa automated nitrogen (N) and carbon (C) analyzer. Cell abundances were quantified by the ODU Phytoplankton Analysis Laboratory following the established Chesapeake Bay Phytoplankton Monitoring Program protocols (Marshall et al., 2005). As a direct response to modeling needs we first examined the relationship between Chl *a* and PC. We also compared the abundance of HAB species during blooms with diagnostic pigment concentrations and Chl *a* to determine the relationships between

these variables and to develop pigment indicators for HABs. Dinoflagellates were the main bloom formers in winter, spring (typically *Heterocapsa triquetra*) and summer (typically *Cochlodinium polykrikoides*) and the marker pigment for dinoflagellates is peridinin (Haxo and Allen, 1960). Therefore, relationships between dinoflagellate cell abundance, peridinin and Chl *a* concentrations were quantified. We also compared peridinin and Chl *a* concentrations with those of other pigments but observed no relationships suggesting that there were no unique indicator pigments for either of the bloom species frequently detected and sampled.

Results

Chl a and PC relationship. For the pooled set of data in 2012, there was a significant ($p < 0.05$) linear relationship between PC and Chl *a* over a range of Chl *a* concentrations (Fig. 2). There was a significant logarithmic relationship between the PC:Chl *a* ratio and Chl *a* concentrations (Fig. 3) showing that the highest PC:Chl *a* ratios were observed when Chl *a* concentrations were lowest (Table 1). The relationship between PC and Chl *a* concentrations is directly relevant to models because Chl *a* is often used to estimate algal carbon (C).

Table 1. Relationship between PC:Chl *a* and Chl *a* concentrations. Standard deviations are in parentheses.

Chl <i>a</i> concentration ($\mu\text{g Chl L}^{-1}$)	PC:Chl <i>a</i> $\mu\text{g C } (\mu\text{g Chl } a)^{-1}$	Sample number
< 5	546.3 (145.1)	14
5 – 10	365.6 (108.4)	85
> 10	162.4 (79.6)	217
> 30	122.3 (47.6)	131
> 50	117.5 (44.8)	114
> 100	115.9 (46.5)	56
0 – 649	234.9 (144.80)	318

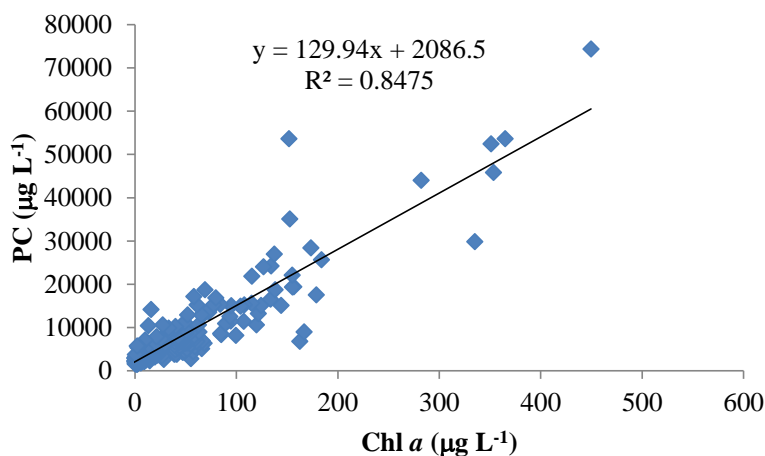


Figure 2. Relationship between particulate carbon (PC) and Chl *a*

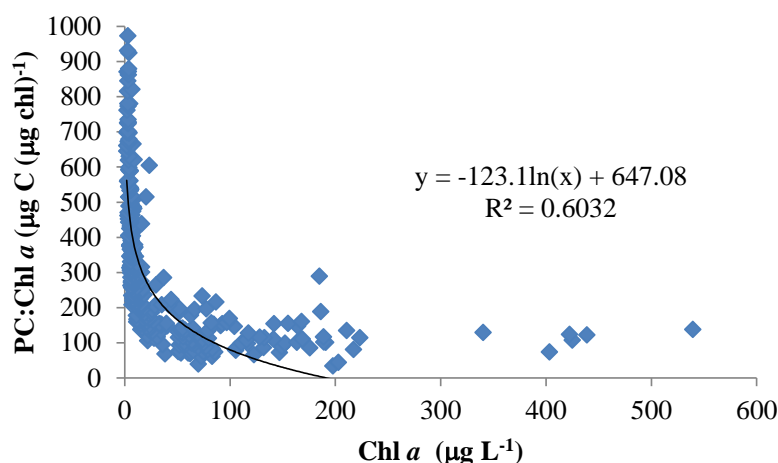


Figure 3. Relationship between PC:Chl *a* and Chl *a* concentrations.

In general, dinoflagellate abundance and peridinin concentrations were significantly ($p < 0.05$) and positively related to Chl *a* concentrations in the Lower James River estuary in 2011 and 2012 even in samples where Chl *a* was $< 50 \mu\text{g L}^{-1}$ (although the relationship was weaker) (Fig.4). Periods of high Chl *a* concentrations were often associated with blooms of *Heterocapsa triquetra* and *Cochlodinium polykridoides*. Significant positive linear relationships ($P < 0.05$) between peridinin and Chl *a* were observed during blooms of *Heterocapsa triquetra* in 2011 in the JMSMH (Fig. 5A). Peridinin and Chl *a* concentrations were significantly ($p < 0.05$) correlated in all river sections during 2012 when sampling was conducted during bloom and non-bloom periods (Fig. 5B) suggesting that elevated chlorophyll concentrations were always due to abundant dinoflagellates.

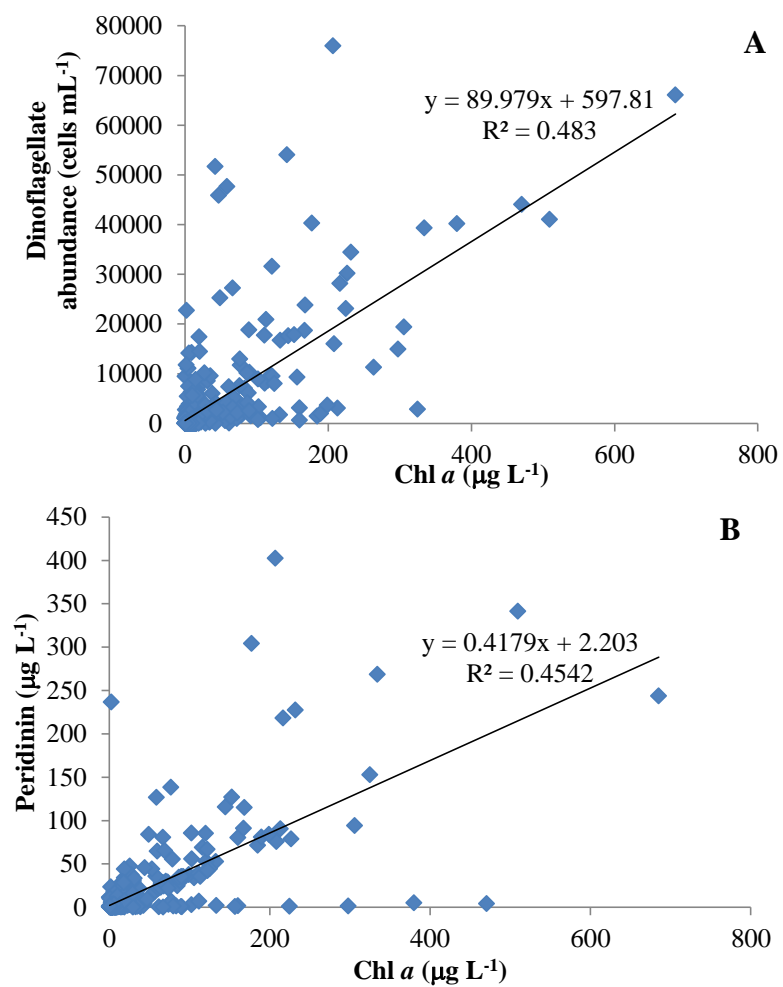


Figure 4. Relationship between Chl *a* and dinoflagellate abundance (A) and the relationship between Chl *a* and peridinin in pooled samples (B) from 2011 and 2012.

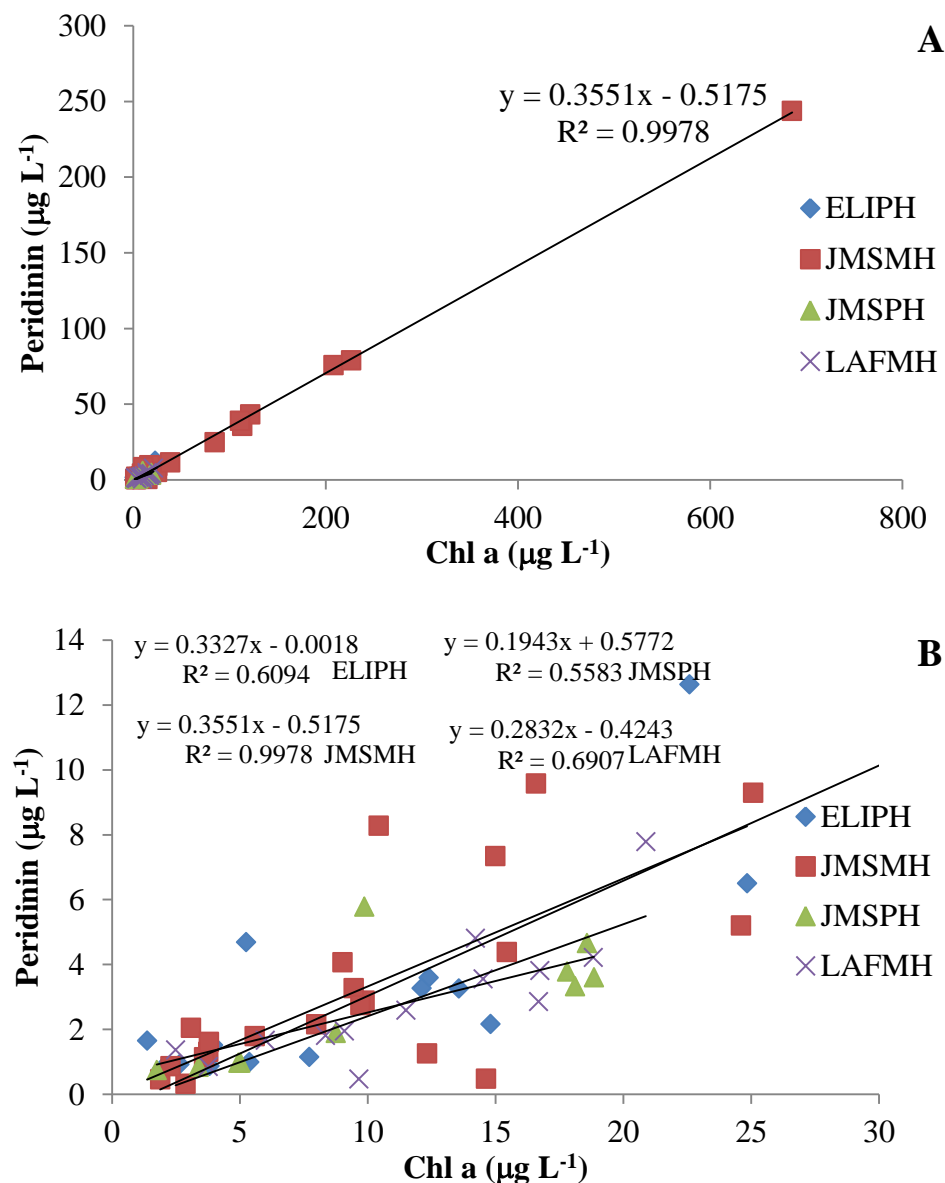


Figure 5. Peridinin vs Chl *a* concentrations ($\mu\text{g L}^{-1}$) for samples collected during a *Heterocapsa* bloom in 2011 (A); and for all samples collected in 2012 during bloom and non-bloom periods in each river segment between March and October (B). The latter excludes samples collected when Chl *a* concentrations were greater than $30 \mu\text{g L}^{-1}$.

Significant ($p < 0.05$) positive linear relationships between peridinin and Chl *a* were observed in the summer months when *Cochlodinium polykridoides* was the dominant dinoflagellate present (Fig. 6), however the R^2 values for the peridinin-Chl *a* relationship in the JMSMH segment was lower ($R^2 = 0.43$; Fig. 6) than that observed during the 2011 *Heterocapsa* bloom ($R^2 = 0.99$; Fig. 5A) or during 2012 bloom and non-bloom sampling ($R^2 = 0.69$; Fig. 5B).

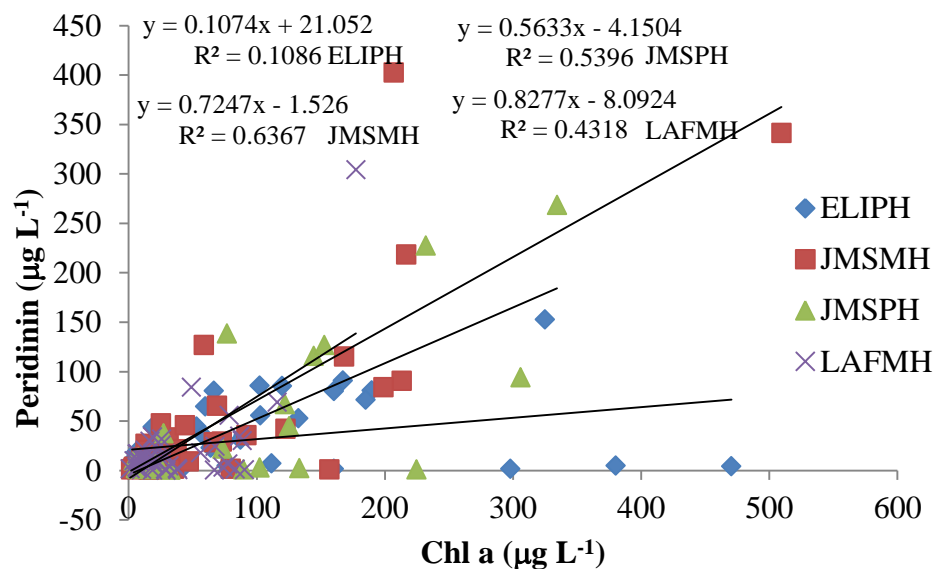


Figure 6. Peridinin vs Chl *a* concentrations ($\mu\text{g L}^{-1}$) for samples collected when *Cochlodinium* was present in the James River in 2011 and 2012 (n=157).

On an annual basis, the relationship between peridinin concentration and *Cochlodinium* abundance varied between years (Fig 7A). In 2012, there were more samples that had *Cochlodinium* present compared to 2011, and in 2011 cell abundance was never greater than 5,000 cells mL^{-1} . During the winter and spring bloom of *Heterocapsa*, significant linear relationships were similar when comparing 2011 and 2012 (Fig. 7B). The ratio of peridinin to Chl *a* also varied by season and between seasons and years. However, there was no consistent trend between this ratio and dinoflagellate abundance (Fig. 8).

While we show here the relationship between dinoflagellate abundance and peridinin, we also measured high concentrations of the yellow pigments (xanthophylls) violaxanthin and diadinoxanthin, during some sampling periods but these were not correlated with total dinoflagellate abundance or the abundance of *Heterocapsa* or *Cochlodinium* so could not be used as biomarkers for these organisms. These pigments may play a role in photoprotection but were unrelated to taxonomic composition of the phytoplankton community.

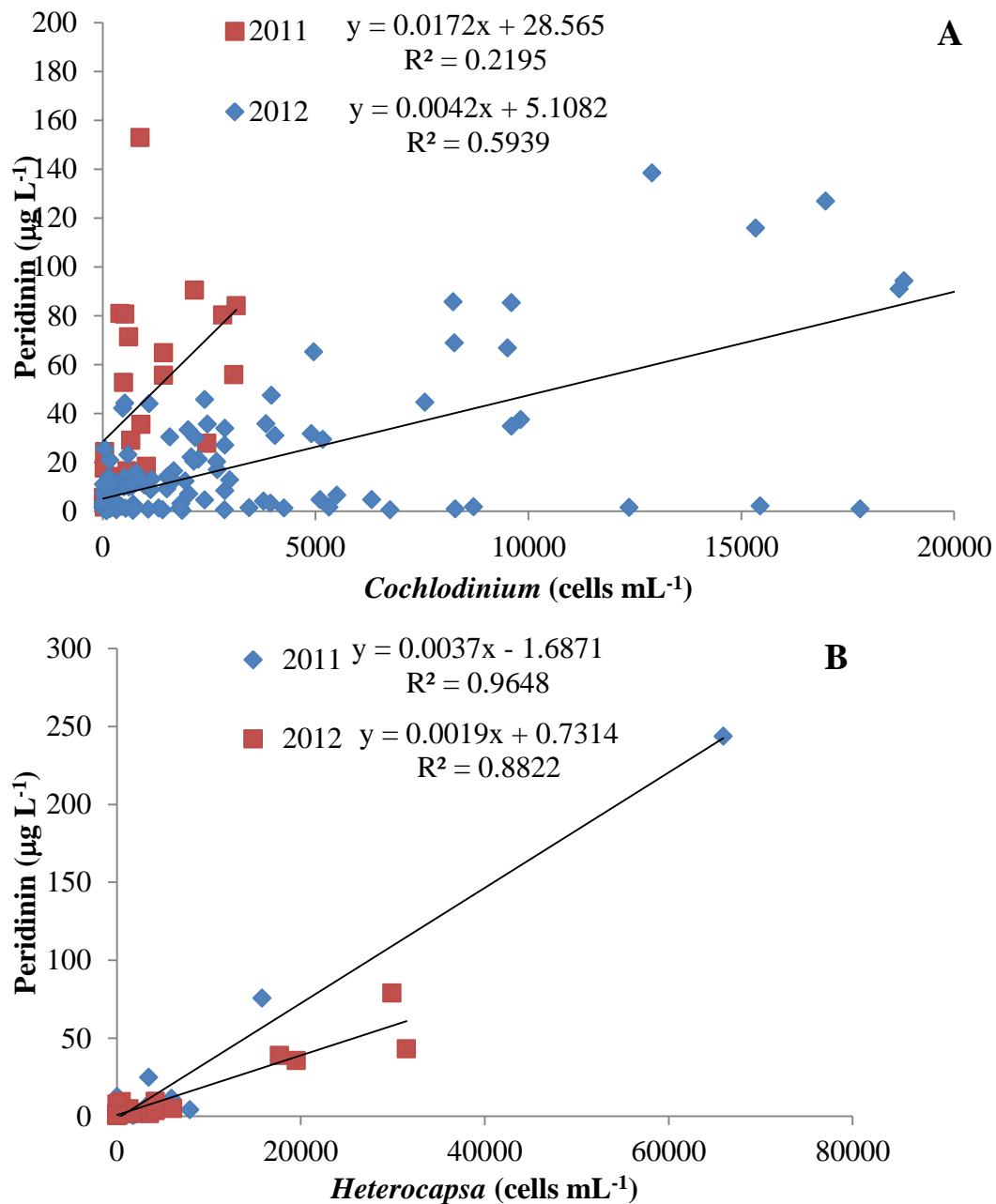


Figure 7. Peridinin vs *Cocchloidium* abundance (A) and *Heterocapsa* abundance (B) in 2011 and 2012 in samples pooled from all river segments.

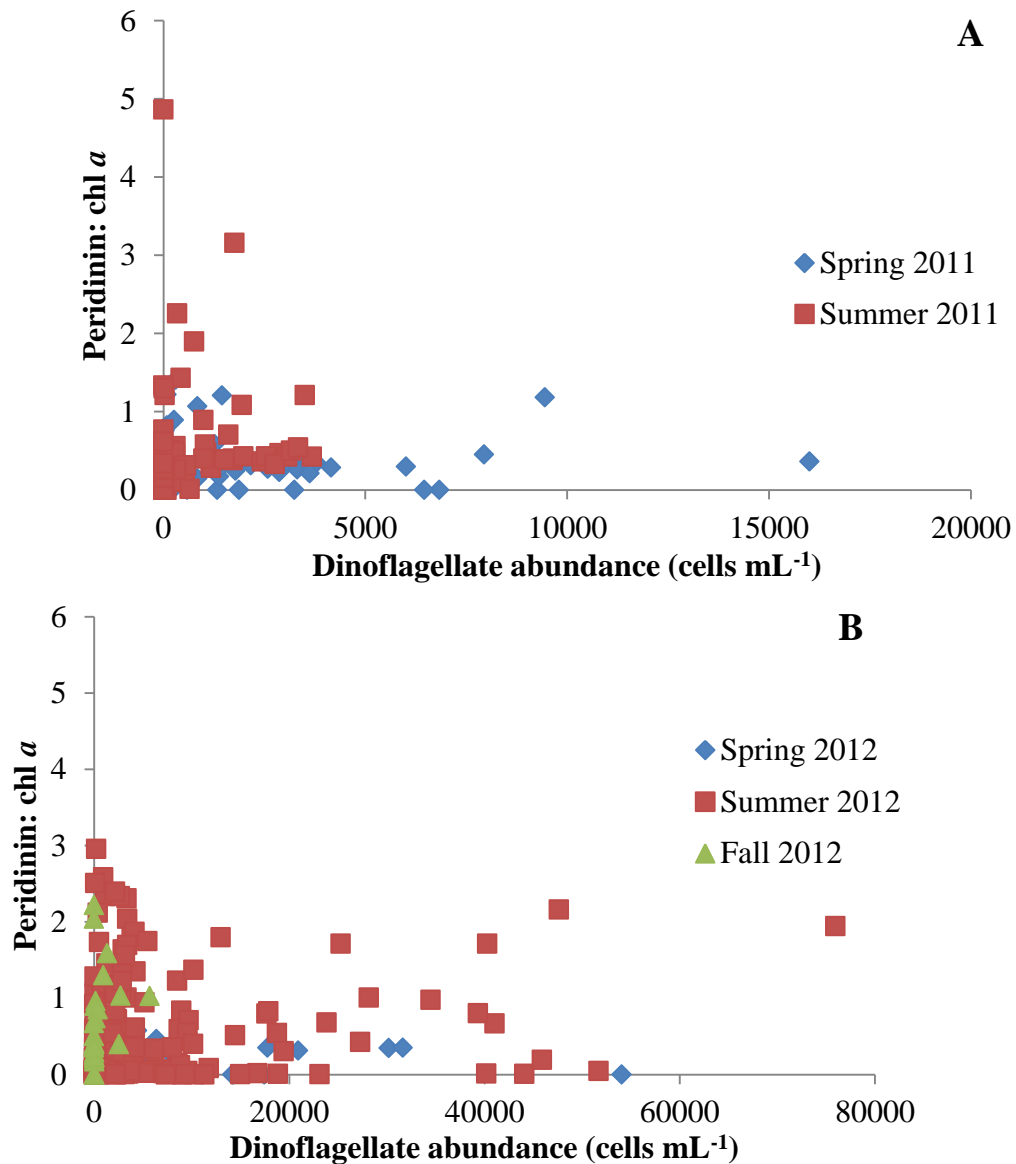


Figure 8. Peridinin:Chl *a* ratio relative to dinoflagellate abundance and distinguished by season in 2011 (A) and 2012 (B).

Discussion

We observed a significant linear relationship between PC and Chl *a* over a range of Chl *a* concentrations (Fig. 2). This is an important relationship for the modeling component of the James River Study because the two metrics of biomass are often assumed to change in constant fashion. However, the C:Chl ratio decreases exponentially with increasing Chl *a* concentrations. This could happen if living C is an increasing proportion of the total particulate C as algal abundance increases. It is important to understand this relationship in order to correctly parameterize the effect of blooms in the environment because it is C and not Chl *a* that generates oxygen demand in the environment.

We observed high concentrations of the dinoflagellate pigment peridinin in association with high Chl *a* concentrations associated with blooms of *Heterocapsa triquetra* and *Cochlodinium polykridoides* during 2011 and 2012. During periods when dinoflagellate abundance was low, peridinin was near the limit of analytical detection. Both dinoflagellate and peridinin concentrations were positively correlated with Chl *a* concentrations in the lower James River estuary (Fig. 4) demonstrating that dinoflagellates were the primary contributor to high Chl *a* concentrations in this river segment. Our results suggest that peridinin concentrations are a good indicator of dinoflagellate abundance in the lower James River estuary as has been shown in other eutrophic estuarine and coastal systems (Paerl et al. 2003, 2010). By monitoring this pigment, we may better predict whether algal populations are likely to potentially harmful dinoflagellate blooms and result in degraded water quality in this river segment.

While we also measured high concentrations of the yellow pigments (xanthophylls) violaxanthin and diadinoxanthin, during some sampling periods, we were unable to relate concentrations of these and other pigments to Chl *a* concentrations so doubt these will be good diagnostic indicators of water quality.

References cited:

- Haxo, F. and M. Allen. 1960. Comparative biochemistry of photoreactive systems. *Acad. Press, NY*.
- Hooker, S. B., L. Van Heukelem, C. S. Thomas, H. Claustre, J. Ras, R. Barlow, H. Sessions, L. Schlüter, J. Perl, C. Trees, V. Stuart, E. Head, L. Clementson, J. Fishwick, C. Llewellyn, and J. Aiken. 2005. The Second SeaWiFS HPLC Analysis Round-Robin Experiment (SeaHARRE-2). NASA/TM-2005-212785.
- Marshall, H.G., L. Burchardt, and R. Lacouture. 2005. A review of phytoplankton composition within Chesapeake Bay and its tidal estuaries. *Journal of plankton research* 27(11): 1083-1102.
- Paerl, H. W., K. L. Rossignol, S. N. Hall, B. L. Peierls, and M. S. Wetz. 2010. Phytoplankton community indicators of short- and long-term ecological change in the anthropogenically and climatically impacted Neuse River Estuary, North Carolina, USA. *Estuaries and Coasts* 33: 485-497.

Paerl, H. W., L. M. Valdes, J. L. Pinckney, M. F. Piehler, J. Dyble, and P. H. Moisander. 2003. Phytoplankton photopigments as indicators of estuarine and coastal eutrophication. *BioScience* 53: 953-964.

Pinckney, J. L., H. W. Paerl, M. B. Harrington, K. E. Howe. 1998. Annual cycles of phytoplankton community-structure and bloom dynamics in the Neuse River Estuary, North Carolina. *Mar. Biol.* 131: 371-381.

Sellner, K. G., S. G. Sellner, R. V. Lacouture and R. E. Magnien. 2001. Excessive nutrients select for dinoflagellates in the stratified Patapsco River estuary: *Margalef* reigns. *Mar. Ecol. Prog. Ser.* 220: 93-102.

Van Heukalem, L., and C. Thomas. 2001. Computer-assisted high performance chromatography method development with applications to the isolation and analysis of phytoplankton pigments. *Journal of Chromatography A* 910: 31-49.